

To Cite:

Borkar SG, Ajayasree, TS, Chavan VA. Spread and Perpetuation of Bacterial Leaf Spot Pathogen *Xanthomonas axonopodis* pv. *mangiferae-indicae* in Mango Orchards. *Discovery*, 2021, 57(303), 192-197

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Peer-Review History

Received: 10 December 2020

Reviewed & Revised: 12/December/2020 to

26/January/2021

Accepted: 28 January 2021

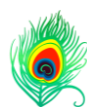
Published: March 2021

Peer-review

External peer-review was done through double-blind method.

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DISCOVERY
SCIENTIFIC SOCIETY

Spread and Perpetuation of Bacterial Leaf Spot Pathogen *Xanthomonas axonopodis* pv. *mangiferae-indicae* in Mango Orchards

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ABSTRACT

Bacterial leaf spot and canker of mango caused by *Xanthomonas axonopodis* pv. *Mangiferae-indicae* is an important disease of mango world-over. In Maharashtra state, India, the disease symptoms appear on the mature leaves of mango plants in the month of Nov-Dec which includes water-soaked irregular lesions ranging from 1-5mm. These lesions are surrounded by a yellow halo. Several lesions are formed on a single leaf. Such infected lesions are transformed into a cankerous spot. The infection also spread to unripe mango fruit causing blemishes and cracks on the fruit. At the end of the mango season, in the month of June, all the infected leaves drop down in the mango basin with the emergence of new leaves. The young new juvenile leaves are not susceptible to the bacterial pathogen and are observed free from bacterial infection up to a period of 5 months. The bacterial pathogen perpetuates in the fallen diseased leaves and also survives as an epiphyte on juvenile mango leaves till it gets mature to succumb to the infection to cause disease. The spread of the disease pathogen is through the air current during rains in June-July. The air current blows at a speed of 26 km/h in the mango orchards which carries the infected dried leaves with it up to a distance of 88 meters in/around the orchard, which disintegrates due to weather and rainy conditions and releases the bacterial pathogen in soil. The disintegrated infected leaves remaining in the plant basin serve as a source of severe infection in the same plant in the next season while the blown-up leaves serve as a source of infection to other plants in the orchard.

Keywords: Bacterial leaf spot and canker, Maharashtra, mango, perpetuation, spread, *Xanthomonas axonopodis* pv. *mangiferae-indicae*

INTRODUCTION

Bacterial leaf spot and canker of mango incited by *Xanthomonas axonopodis* pv. *mangiferae-indicae* is an important disease of mango in several mango growing countries particularly of subtropical and tropical regions, North, South and Central America, the Caribbean, Oceania, Australia, New Caledonia, Africa, and Asia (Borkar, 2017). Losses can be serious if environmental conditions are favourable (Pruvost et al. 2011). The trees may weaken through the loss of foliage,

which eventually leads to lower yields (Kotze and Visser, 1977, Irfan et al. 2017) and canker infected fruits.

In India, the disease was first reported by Patel et al. in 1948. Prakash et al. (1994) reported it as a serious disease of mango in most of the mango-growing states in India. They reported it to be more severe in the month of May, whereas in winter (Nov to March) it remains dormant. In mango, the bacteria may be present epiphytically on juvenile tissues without visible symptoms (Pitkethley, 2006). The bacteria then gain access through stomata or wound where they become endophytic and eventually cause disease (Pruvost et al. 2009). The disease appears year after year due to the perpetuation and spread of the pathogen in the mango orchard and far off places due to transportation of infected mangoes and infected leaves for religious purposes. The knowledge of the spread and perpetuation of this disease, which is not available at present, shall be useful in devising its control strategies, wherever the disease is present. The present investigation reports the results on the spread and perpetuation of the bacterial pathogen in the mango orchards in Maharashtra, India.

MATERIALS AND METHODS

Studies on the perpetuation of *X. a. pv. mangiferae-indicae* in infected leaf sample of mango

The *X. a. pv. mangiferae-indicae* infected leaf samples fallen in the basins of the mango orchard were collected to study the perpetuation of the bacteria in the leaves. The infected leaf samples were collected at different time intervals from the mango basins and these were subjected for the isolation of the bacterium by employing routine plant pathogenic bacterial isolation method (Borkar, 2018) on nutrient agar medium to note down its perpetuation in the fallen mango leaves. Similarly, the mango basin soil was also assessed for the presence of the bacterial pathogen at different time intervals by its plating on nutrient agar medium by following a serial dilution method of soil samples. The isolated bacterium was proved pathogenic on mature mango leaves of 2 years old plant by syringe inoculation technique (Borkar, 2018) which produced typical leaf spot symptoms within 8 to 10 days (Robbs et al. 1974).

Persistence of Epiphytic population of *X.a.pv. mangiferae-indicae* on juvenile mango leaves

The persistence of the epiphytic population of *X.a.pv. mangiferae-indicae* on mango leaves were studied by employing the leaf imprint method (Yumlembam, 2016). The juvenile leaves were sprayed with a bacterial suspension of *X.a.pv. mangiferae-indicae* (10^8 CFU/ml) and the leaf imprint was taken of such sprayed leaves at fortnight and thereafter at monthly intervals up to 6 month, i.e at the maturity of the leaf. The presence of *X. a. pv. mangiferae-indicae* was noted in the leaf imprinted nutrient agar plates as epiphytic colonies in the absence of any leaf spot development. Such epiphytic colonies of *Xanthomonas* that appeared on the mango leaf imprinted plates were tested for its pathogenicity on mature mango leaves. The development of the symptoms on mature mango leaves was a confirmation of the pathogenic nature of the *Xanthomonas* colonies; while on the juvenile mango leaves its presence was considered as epiphytes (in the absence of development of symptoms on the juvenile leaves).

Dispersal of the infected leaves through wind currents

The dispersal of bacterial leaf spot disease leaves from the infected mango plants was studied by measuring the distance of infected mango leaves carried over by the wind currents to other places in the orchard. Such infected leaves dispersed through wind currents from the mango basin to a particular point/distance were collected, the dispersal distance was measured, and subjected for the isolation of bacterial pathogen on nutrient agar media to note down the dispersal of the bacterial pathogen through leaves. Similarly, when such dispersed leaves disintegrate during the rainy season to release the bacterium in the soil was also collected along with the soil sample and assessed for the presence of the bacterial pathogen as mentioned above.

RESULTS

In Maharashtra, the bacterial leaf spot and canker of mango is emerging as an important disease. In this region, the disease symptoms appear on the mature leaves of mango plants in the month of November-December which includes water-soaked irregular black lesions ranging from 1 mm to 5mm. The lesions are surrounded by chlorotic yellow halos. Due to vein limitations, the spots become angular and resulted in cankerous, rough, necrotic raised lesions. Several lesions are formed on a single leaf (Fig. 1). The infected lesions are transformed into a cankerous spot. The infection also spread to unripe mango fruit causing blemishes and cracks on the fruit (Fig.2).

The severely infected leaves fall off the plants at the end of the mango season, in the month of June with the emergence of new leaves and such infected leaves remain in the mango basin as dried infected leaves till the beginning of the rainy season (Fig.3). During the rainy season, such fallen infected leaves get mixed with mango basin soil to decompose and release the bacterial

pathogen in the mango basin ecosystem. The bacterial pathogen survives in the fallen infected leaves/mango basin soil up to a period of 6 and 9 months respectively (Table 1).



Fig.1. Bacterial leaf spot symptoms on mango leaves

The isolated *X.a.pv. mangiferae-indicae* from mango basin fallen leaves/basin soil, when tested on the mango plant leaves indicated that the younger newly emerged leaves did not develop the bacterial leaf spot symptoms while the older mature leaves developed the bacterial leaf spot symptoms with a period of 8 to 10 days (Table 2).

Thus, the juvenile and young new leaves of 4 month age were not susceptible to the bacterial infection. However, the bacterium survives on these juvenile leaves as an epiphytic population for around five months or till the leaf become mature to succumb to the infection (Table 3). Thus the bacteria perpetuate on the infected diseased fallen leaves in a mango basin and on the juvenile young leaves as epiphytes.

The spread of the bacterium is through air current. The air current in this region during the leaf fall period (May-June) blows at a speed of 26km per hour which takes the air blown infected dried leaves up to a distance of 88 meters from the infected plants in/around the mango orchard, which disintegrates in rainy weather during the month of July-August and releases the bacterial pathogen in soil. Such air blown disintegrating leaves mixed with soil to release the bacterial pathogen *X.a.pv. mangiferae-indicae*.



Fig.2. Bacterial leaf spot infection-induced cracking on mango fruit



Fig. 3. Bacterial leaf spots infected leaves fallen in mango basin.

Table 1. Survival of *X. a. pv. mangiferae-indicae* in the mango soil basin.

Leaf fall samples in mango basin	Survival period of <i>X. a. pv. mangiferae-indicae</i> (in months)				
	2	4	6	8	9
1. In fallen leaves	+	+	+	-	-
2. In basin Soil	+	+	+	+	+

+ = presence of bacterium, - = bacterium not detected

Table 2. Pathogenicity of *X.a.pv. mangiferae-indicae* on mango foliage.

Mango foliage leaf inoculated	Symptoms developed
1. Tender new juvenile leaves	Nil
2. Young leaves of 3 month age	Nil
3. Old mature leaves of 5 month age	Leaf spot symptoms developed within 8 to 10 days

Table 3. Survival of *X.a.pv. mangiferae-indicae* as leaf epiphytes on mango leaves.

Leafage after new foliage emergence	Presence of bacterium as epiphytes
1. Fortnight	+
2. One month	+
3. Two months	+
4. Three months	+
5. Four months	+
6. Five months	Leaf infection and development of leaf spot symptoms
7. Six months	Leaf infection and development of leaf spot symptoms.

DISCUSSION

Bacterial leaf spot and canker of mango incited by *Xanthomonas axonopodis* pv. *mangiferae-indicae* is an important disease of mango wherever the mango crop is grown (Borkar, 2017). The symptoms produced by this bacterium on mango leaves includes water-soaked irregular lesions ranging from 1-5 mm and surrounded by a yellow halo. Several lesions are formed on a single leaf. Such infected lesions are transformed into a cankerous spot. The infection also spread to unripe mango fruit causing blemishes and cracks on the fruit. Similar disease symptoms are reported from other parts of the world (Robbs *et al.*, 1974; Irfan *et al.*, 2017). The bacterium survives as epiphytes on the young juvenile leaves in the mango orchard till the leaf get mature to succumb to infection. Similar results were also reported by Pitkenthley (2006). The bacterium not only survives as epiphytes on mango leaves but also on the leaves of weeds plant available in and around the mango orchard. Kishun and Chand (1994) reported an epiphytic population of *X. Axonopodis* pv. *mangiferae-indicae* on 18 out of 36 weeds common in and around mango orchards to serve as a source of a new infection.

In our studies, the bacterial leaf spot infected fallen leaves remain in the basin of the mango plant, where it dries and mixes with the orchard soil with the arrival of rains in June-July. With the arrival of rain and disintegration of diseased leaves, the bacterium

gets released and land on the juvenile young leaves to survive as epiphytic population till such leaves mature to succumb to infection. Thus, the bacterium perpetuates and survives on the infected fallen leaves and on the juvenile young leaves as a source of infection. For the spread of the bacterium the air current plays an important role.

The air current in this region during the leaf fall period (May-June) blows at a speed of 26km per hour which takes the air blown infected dried leaves up to a distance of 88 meters from the infected plants in/around the mango orchard, which disintegrates in rainy weather during the month of July-August and releases the bacterial pathogen in soil. Such air blown disintegrating leaves mixed with soil yielded bacterial pathogen *X.a.pv. mangiferae-indicae* upon isolation of the pathogen. Such bacterial pathogen release during the disintegration of the infected leaves in the rainy season is carried to the infection loci by the rain splashes to cause infection (Fitt et al. 2003). Thus, the disintegrated infected leaves in the plant basin serve as a source of severe infection in the same plant in the next season in the month of Nov-Dec while the blown-up leaves serve as a source of infection to other plants in the orchard.

CONCLUSION

Perpetuation and dispersal of bacterial leaf spot pathogen of mango play an important role in the recurrence of infection in mango orchards. The infected fallen leaves have to be collected before the onset of monsoon and should be burnt to minimize the bacterial population load for the initiation of new infection in the next season. Similarly, the mango basin soil should be sprayed with suitable bactericide along with the mango plants at the onset of the rainy season to minimize the inoculum load for recurrence of infection.

Funding:

This study has not received any external funding.

Conflict of Interest:

The authors declare that there are no conflicts of interests.

Peer-review:

External peer-review was done through double-blind method.

Data and materials availability:

All data associated with this study are present in the paper.

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